BACTERIOLOGIC ASPECTS OF CHRONIC PULMONARY EMPHYSEMA

by

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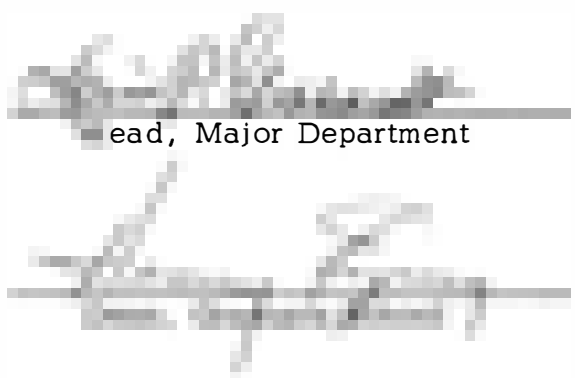

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INTRODUCTION

Definition of Pulmonary Emphysema. One of the oldest crippling diseases known to mankind and one of the major causes of death in middle-aged men in this country is emphysema. The word, emphysema, is derived from Greek, Εμφύσημα or emphysan, which means to blow into, to inflate. The disease emphysema is a distention of alveolar sacs without complete expulsion of expiratory airflow. Diffuse obstructive emphysema has been described by Mitchell (1959) as a chronic, usually progressive, disorder of the lungs which may accompany other lung diseases, but frequently occurs alone. He further classified emphysema as pathological, physiological, roentgenographical or clinical. Pathologically, emphysema was characterized by dilated, thinned, and often ruptured alveoli, bronchiolar obliteration and ectasia, bullae (especially on the surface of the lungs), fibrosis, impairment in pulmonary vasculature, right ventricular hypertrophy, and often dilatation. There is no general agreement on the findings of clearcut gross or microscopic evidence of the airway obstruction so predominant clinically. Physiologically, emphysema was categorized by an absolute and relative increase in residual volume, with increased work of breathing, marked dimunition in ventilation, gas mixing, diffuse capacities, and later evidence of pulmonary hypertension. Roentgenographically, emphysema was noted by large bright lung fields with a paucity of peripheral vasculature markings, increased anteroposterior diameter

of the chest, high clavicles, "squared-off" apices, low flattened hemidiaphragm with impaired excursion, occasional bullae, impaired ventilation (noted particularly on expiration by fluoroscopy), and right heart enlargement. Clinically, emphysema was characterized by dyspnea on exertion unexplained by other findings, a frequent but not invariable history of cough prior to the onset of dyspnea, interference with movement of air out of and, to a lesser extent, into the lungs (usually but not always a progressive course), and the frequent ultimate development of right heart failure.

THE PROBLEM

Statement of the problem. The purpose of this study was (1) to investigate the bacterial flora of patients with emphysema, (2) to see if there was a species or strain of organisms peculiar to emphysema, (3) to test these bacteria for their ability to multiply in pulmonary mucus in vitro, (4) to examine the surface tension properties of mucus in vitro; and (5) to examine the alterations of surface tension of culture media produced by microorganisms.

Importance of this study. The importance of this study was to define some of the basic bacteriologic aspects of emphysema. This basic information may aid in the treatment of patients with early bronchitis or bronchiectasis.

REVIEW OF THE LITERATURE

Theories of the pathogenesis of pulmonary emphysema. The disease, emphysema, is so confusing and complex that the etiology as well as pathogenesis are still unknown (McLean, 1956). In the study of the pathology of emphysema, McLean (1956, 1957a, 1957b, 1957c, 1958a, 1958b, 1959) examined lungs of patients with early stages of this disease. The lungs of patients who died following severe bronchiolitis of one or two months' duration were studied. In this examination, he observed that the bronchioles were completely occluded with early granulation tissue and that the smallest bronchioles were most often affected. The more distal passages were not affected and remained aerated by collateral ventilation. In lungs with lesions of longer duration and in bronchiectatic lungs, the bronchiolar remnants were inconspicuous. He found bronchioles which ended blindly. The remnants of the obliterated bronchioles could not be recognized. There was sufficient evidence for the presence of large bronchioles, but alteration in architecture and tissue were such that passages of the size of respiratory bronchioles left no discernible traces of their original structure. Similar examinations which yielded comparable results were done with emphysematous lungs. Once again remnants of large bronchioles were identified with certainty but search for remnants of smaller bronchioles was frustrating. Grossly, the emphysematous lungs revealed much destroyed lung tissue. The alveoli bearing passages, the larger bronchioles, and the blood vessels

of corresponding size were destroyed without leaving conspicuous remnants. In the histologic study of the clinical emphysematous lungs, the structure was found to be so greatly altered that few clues were evident which might indicate the sequence of pathological changes. The pathological changes in the emphysematous lungs suggested to McLean (1958a,1959) that the primary lesion was probably located in the respiratory bronchioles. He studied lungs of patients with minimal centri-lobular emphysema and discovered that the periphery of an affected lobule was normal. Centrally there was considerable destruction of air passages involving both the musculo-elastic mesh forming the true walls and the walls of the associated alveoli. McLean (1959) reported that in minimal emphysema the air passages involved can be recognized as respiratory bronchioles which have lost many of their alveoli, leaving only irregular remnants of the musculo-elastic mesh. In more advanced lesions, McLean could not positively identify the respiratory bronchioles. The most distal bronchiole, from which numerous intercommunicating pathways arose, forms what has been termed a "common pool" (Leopold and Gough, 1957 and McLean 1959). From the "common pool" relatively normal air passages continue to the periphery of the lobules.

McLean (1958a, 1959) observed that there was evidence of inflammatory damage found regularly in the tissues in emphysematous

regions and in immediately proximal bronchioles. He also noticed an increased deposition of collagen and reticulin, patchy destruction of special tissues, and partial obliteration of the lumen of an occasional bronchiole. This evidence of past bronchiolar inflammation led McLean to the study of homeostasis in the bronchial tree. Homeostasis is defined as equilibrium of fluid content, chemical reaction and temperature. McLean stated that the establishment of bronchiolitis largely depended on defective homeostasis. He reported that homeostasis depends on "ciliary action", cough, and collateral ventilation, all of which are primarily concerned with the maintenance of a patent airway. Mucus or any fluids in the bronchial tree are ordinarily swept, as a continuous sheet up to the larynx and pharynx. He stated that overloading of this mechanism results in the formation of masses of mucus which may be expelled by coughing or may be aspirated deep into the bronchial tree, producing complete occlusion of bronchioles. McLean (1958a, 1959) theorized that this probably occurs in healthy persons frequently, but with no ill effect, because the lung distal to the occluded bronchiole is collaterally ventilated from adjacent lung tissues through the alveolar pores, and a subsequent cough soon expels these plugs. He suggested that excessive mucus or exudate in the respiratory tract may lead to defective homeostasis. The mechanisms of homeostasis may be directly affected by the following: (1) Weak

coughing, (2) Destruction of cilia, (3) Occlusion of alveolar pores by exudate or, (4) Reduction in collateral ventilation due to shallow respiration. In such circumstances he suggested that bronchiolar occlusion occurs more often and the plugs tend to remain in situ for relatively long periods. McLean (1958a, 1958b) speculated that the most common cause of breakdown of homeostasis was respiratory infection caused by common viruses. He believed that bronchiolar plugging occurred freely during such episodes, particularly at night when breathing was quiet and cough was suppressed. Frequently these mucous plugs in the bronchioles may contain bacteria. If expulsion does not occur rapidly, these bacteria may proliferate and might reach a concentration within the plug sufficient to produce an inflammatory reaction in the adjacent bronchiolar wall. In very serious bronchiolar infections, McLean discovered innumerable plugs of this type with leukocytes traversing the mucosa and accumulating, at first, on the periphery of the plugs. He emphasized that mucous plugs which remained present for a prolonged time in the respiratory tract may allow bacteria to multiply. For this reason, he thought that bacterial inflammation in the respiratory bronchioles occurred at the site of maximal bronchiolar plugging in the small bronchioles, that inflammation of this type tended to persist as long as homeostasis remained defective, and that viral infections were a causative factor in bronchiolar damage

and in predisposing to bacterial bronchiolitis. He suggested that "bacteria of low pathogenicity" were a major cause of bronchiolar inflammation and damage because of their potentially long duration of action. He reported that once "bronchiolar inflammation" was established, two courses may follow: First, the plug may be expelled; damage to wall structures may occur but, because of regeneration of normal tissue the microscopic examination obtained later may appear normal. Secondly, the plug may be retained and the lumen may become partly or completely occluded. McLean's theory of the pathogenesis of emphysema is controversial. Many experienced pathologists deny the presence of these bronchiolar lesions, but agree to the pathological changes in the centrilobular areas.[1] Most pathologists find no evidence of bronchiolar obstructions in this disease (Berthrong 1959, Gordon 1959, and Gaensler, 1959).

Segal and Dulfano (1953) were not sure whether or not bacterial infection was the primary cause, but they felt it played a definite role in the pathogenesis of emphysema in that infection may act in producing auto-immunological sensitization. Read (1958) demonstrated a series of pulmonary changes characteristic of pneumonotoxic pneumonia in rats by injecting rabbit anti-lung serum intra-tracheally. Crowle (1959) found that inhalation of chemically reactive fumes,

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[1] Statement by Dr. William Harris, Women's Medical College, Philadelphia, Pennsylvania, personal interview.

or large volumes of tobacco smoke could slightly injure the lung tissue. This slightly injured lung tissue could make it foreign antigenically to the victim's immunologic processes. He suggested that molds, spores, and bacteria associated with chronic lung diseases could function as adjuvants. This small amount of injured tissue in proximity to an adjuvant, may cause auto-immunization. If the affected lung tissue happened to be elastic tissue, for example, emphysema might be its most obvious manifestation. In attempts to prove this hypothesis, Crowle (1959) injected three guinea pigs subcutaneously with Freund's adjuvant containing a guinea pig lung homogenate. The homogenate was prepared from lungs of guinea pig killed slowly by nitrous oxide gas meant to injure its lungs to change slightly the chemical constitution of the lung protein. Approximately 3 weeks after their treatment, all began breathing abnormally. After 5 weeks, 2 of the 3 animals died and the third was killed at the same time. The lungs of the two animals which died were larger than normal, were bright red instead of normal flesh color and appeared to retain more air than normal guinea pig lungs. About one-third of the killed guinea pig's lungs were involved similarly, but with no apparent order or distribution. The disease was less acute, but quite definite. The study of antibodies to human lungs in patients with chronic emphysema and pulmonary tuberculosis was investigated by Hennes et al. (1961). Their

results suggested that tubercle bacilli may serve as an "endogenous Freund adjuvant" stimulating antibody response to damaged tissue. They have concluded from the absorption studies that these antibodies are directed against different components of the lung antigen and that antibodies in the serum from patients with tuberculosis and emphysema differed. The antibody in emphysema was absorbed by the supernatant from tryptic digestion of the lung while the antibody in tuberculosis is not.

Another theory for the pathogenesis of emphysema has been reported by Lowell et al. (1956). These investigators studied thirty-four patients with chronic disease characteristic of obstructive emphysema. The ages of these patients ranged from 50 to 81 years. There were 28 males and 6 females. From this study smoking was the major cause of emphysema and that the disease was inflammatory rather than degenerative in New England area residents.

Segal and Dulfano (1953), McLean (1958a, 1958b, 1959), Krahl (1959), Gaensler and Lindgren (1959), and Fletcher (1959) believed that emphysema was the end result of repeated mild bronchiolar infections. If emphysema is the end result of chronic bronchitis, the study of the causes of chronic bronchitis is necessary. Mulder and Hers (1953) reported that 9 cases of acute and chronic muco-purulent bronchitis were associated with nonencapsulated Hemophilus influenzae infections. Knox and Elmes (1955) studied groups of patients with chronic bronchitis during acute exacerbations

of this disease. These authors noted a high incidence of H. influenzae or Diplococcus pneumoniae in sputum from their patients. They observed that when there was a proliferation of one of the pathogens, usually either D. pneumoniae or H. influenzae the sputum became purulent. During antibacterial therapy the number of pathogenic bacteria and inflammatory cells were reduced in number and the sputum appeared normal. Oswald (1957) examined macroscopic features of sputum of 1,000 patients with chronic bronchitis. The sputa were collected in the summer, winter and during exacerbations. In his studies H. influenzae and D. pneumoniae were the most frequent pathogens isolated from sputa containing pus. The sputa were examined before and after administration of various antimicrobial drugs and notation made of which organisms were related to the presence or absence of pus. Douglas et al. (1957) studied the response to antibacterial therapy in 131 patients with exacerbations of chronic bronchitis and bronchiectasis. "Surgical 'Dettol'", a colorless liquid which does not precipitate protein, was used in the sputum containers and the floating contents were classified into one of four grades: (1) purulent, (2) mucopurulent, (3) containing a trace of pus, and (4) mucoid. The degree of infection during the courses of treatment was estimated by the microscopic appearance of the sputa. H. influenzae and D. pneumoniae were predominate organisms found in purulent, mucopurulent sputa and sputa containing

a trace of pus. Hallet et al. (1959) reported that there was an association between the presence of purulent or mucopurulent sputum and predominance of pneumococcus.

McLean (1958a, 1958b) observed microscopically evidence of bacterial proliferation in mucous plugs. Therefore, it was necessary to examine previous studies of the effect of mucus or mucus-like substances on the growth and virulence of bacteria. One of the earliest investigations of the effect of mucin on bacteria was reported by Nungester and Jourdonais (1932, 1933, 1935, 1936). They observed that pneumococci injected intra-tracheally, intra-bronchially or intra-peritoneally with sterile gastric mucin produced lobar pneumonia in experimental animals. These authors were able to demonstrate different stages of lobar pneumonia in rats and mice similar to stages observed in human. When pneumococci suspended in saline were inoculated into animals, the incidence of pneumonia was strikingly lower. Further studies on the effect of mucin and bacteria on experimental animals have been reported by Rake (1934, 1935). Gastric mucin containing small numbers of Salmonella typhosa, injected intra-peritoneally into mice produced disease more consistently than did larger inocula in broth. Olitzki and Koch (1945) showed different degrees of effectiveness in enhancing the pathogenicity of intra-abdominally injected Shigella dysenteriae in rats by using two mucin preparations of different origin. They observed

that the active mucin (N) contained more of an insoluble fraction rich in inorganic substance, mostly aluminum than the less effective mucin preparation (W). They found that the activity of the pathogenicity-enhancing substance was inversely proportional to the rapidity of their elimination from the abdominal cavity. Long persistence in the abdominal cavity, ability to adsorb and destroy white cells and, as a consequence, to prevent phagocytosis were the important properties for the effect of pathogenicity-enhancing substances.

Radford studied pressure-volume relationships of isolated lungs and have reported unusual surface tension properties. He found evidence of a remarkably active surface tension reducing substance in pulmonary tissue. This surface tension reducing substance was found in lung extracts and in bronchial mucus. This important substance must be present in order that the dynamic forces of inspiration move air to the alveoli. He irrigated the isolated lungs with normal saline. This removed the surface tension reducing substance. The lungs devoid of surface tension reducing substance showed that a marked increase in inspiratory pressure was required to produce airflow to the alveoli. He believed that elevated surface tension at the bronchiolar and alveolar interfaces was responsible for this increase in inspiratory pressure. When surface tension reducing substance from prepared normal lung tissue

was added to the irrigated lungs, the normal pressure volume relationships were restored.[2]

Chemical alteration of normal bronchiolar mucus could destroy the surface tension reducing properties of mucus. This destruction in the surface tension properties of mucus may cause the bronchiolar wall to lose its elasticity and in turn the contractive force may be lost which may cause bronchiolar obstruction. In this event, the larger bronchi and bronchioles may be sufficiently rigid to remain open. However, the smaller bronchioles with less rigid walls might become occluded by the elevated surface tension alone. It is possible, as theorized by McLean that the centrilobular destructive changes of emphysema follows bronchiolar obstruction.

If bacteria proliferates in the bronchial mucus, and if this bacterial growth alters or destroys the surface tension reducing properties of mucus, bronchiolar obstruction could occur. The obstructed bronchioles in turn may inhibit bacterial growth due to the defective airflow. Thus, in such cases mucous secreting activity of the affected bronchioles may be restored to normal. With restoration of normal bronchiolar mucus, the surface tension reducing substance is again present, and the bronchiole becomes patent. This then, might explain the centri-lobular destructive changes in the presence of a patent bronchiole when the lung is

[2] Dr. Edward Radford, personal interview with Dr. William Harris.

examined pathologically. The obstruction of the bronchioles may be dynamic and reversible depending upon the concentration of the surface tension reducing substance at various times.

Viral studies of the upper and lower respiratory tract. That viral infections are important in predisposing to pulmonary bacterial infection was reported by Mulder and Straub (1948), Mulder and Verdonk (1949), Mulder (1952) and McLean (1958a, 1958b, 1959). For example, Mulder and Verdonk (1949) isolated influenza A virus from a patient dying of tracheobronchitis and bronchopneumonia due to Staphylococcus aureus. They found in this patient's trachea and right bronchus, besides acute fibrinopurulent inflammation, very peculiar degenerative and regenerative epithelial changes with severe hyperemia and mononuclear infiltration of the mucosa. They believed that these epithelial lesions were due to the influenzal virus and not to the staphylococcal toxin.

Evans (1957) reported that over 2,000 students at the University of Wisconsin report yearly to the student clinic. Respiratory infections were the single most important cause of admission to the infirmary, accounting for almost a third of all admissions. The highest incidence of respiratory infections occurred in January, February, and March. Non-bacterial infections out-numbered bacterial infections 5 to 1 and were responsible for 84% of all respiratory illness. Evans (1957) studied the causes of acute

respiratory diseases in 290 University of Wisconsin students admitted to the infirmary. The identification of viruses was made with the use of serologic techniques and HeLa cell cultures. Total of 16.9 percent known infections was established. These infections included the following: adenovirus infection 2.0 percent, streptococcal tonsilitis and pharyngitis 8.3 percent, influenza 3.4 percent, herpetic infection 1.3 percent, bacterial pneumonia 1.6 percent, and psittacosis 0.3 percent. A total of 83.1 percent were of unknown causes.

The etiology of common respiratory infections in a civilian adult population was investigated by Grieble et al. (1958). One hundred and twenty-two acute respiratory illnesses among civilian adults in clerical, technical or professional positions in a large city were investigated by clinical and laboratory methods. Ten of them were caused by influenza viruses, 7 by beta hemolytic Streptococci, 4 adenoviruses, 2 were serologically compatible with infectious mononucleosis and 6 were diagnosed clinically as coxsackie virus infections. Approximately one-fourth of the group of patients had fever. Sixty three were afebrile illnesses of unknown etiology in which acute coryza or sore throat were the predominant symptoms. Concomitant fever, pharyngeal exudate or conjunctivitis was present in the 27 cases of pharyngitis. Seventy-five percent of the infections were caused by unknown or undetected agents. Known viruses or

diagnosable infections presumed to be viral occurred in 19 percent and beta hemolytic Streptococci occurred in 6 percent.

Chanock et al. (1958) recovered 41 agents in monkey kidney tissue cultures from throat swabs of infants and young children with respiratory illnesses during October and November 1957. Three of the isolates were influenza A, Asian type, whereas the remaining 38 were not neutralized by standard influenza antiserum used for typing. These viruses which were not typable as influenza viruses were identified as hemadsorption viruses, Type 1 and Type 2. Type 1 hemadsorption virus was isolated from 35 children, 8 of whom were studied in the hospitals and 27 whom were involved in an outbreak of febrile respiratory illness in the nursery. The Type 2 hemadsorption virus was isolated from 3 children hospitalized with croup. Identification of Type 1 and Type 2 strains gave a higher titer with chicken red blood cells.

Another viral study has been reported by Pereira and Pereira (1959). They were able to isolate Coe virus from 4 patients with upper respiratory diseases. This virus was isolated readily in HeLa cell cultures but failed to grow in monkey kidney cell cultures. These investigators stated that the incidence of infection with this virus in Great Britain is low in children and rises steadily with ages, and that antibodies are commoner in males, especially among young adults.

Further evidence of viral studies in acute respiratory disease has been reported by Hamre et al. (1961). Throat swabs and acute and convalescent serum specimens were collected between October 1, 1958 and June 1, 1959 from 228 adults and 9 children. Thirty seven of the 237 throat swab specimens yielded viruses. Forty-three cases were known virus implicated. Influenza A and B accounted for 29 illnesses, adenovirus, parainfluenza 1 and 2 and chimpanzee coryza agent were isolated in 14 instances.

Viral studies from common colds were performed by Tyrrell et al. (1961). Washings were mainly collected from patients in the acute phase of typical common colds, of varying degrees of severity. The specimens were cultured in human embryo kidney and rhesus monkey kidney cells. These investigators were able to isolate cytopathic viruses from 25 nasal washings. Nine of these viruses apparently caused colds in human volunteers. Five washings from which cytopathic viruses were not isolated were shown to cause colds in volunteers. In one of these viral studies, nasal washings were collected from a patient during 4 colds, which occurred in a 3 year period. These washings contained 3 distinct agents, 2 of which were cytopathic. These authors suggested that there is some evidence that different agents cause colds with slightly different clinical symptoms and induce the production of specific neutralizing antibodies. Buckland et al. (1961) investigated 43

volunteers of both sexes, ages 18-45 years. These volunteers were inoculated with ECHO virus type 20. Twenty seven volunteers became ill. The main symptoms and signs observed were headache, malaise, aching limbs, sore throat, and fever. Two volunteers had symptoms resembling the common cold and 8 had abdominal symptoms. Viruses were readily found in the throat and feces of most volunteers and the antibody responses of fourfold or greater magnitude occurred in 20 or 25 volunteers from whom viruses were recovered.

Disney et al. (1960) investigated 2 epidemics of bronchiolitis in infants during the winter of 1955-1956 and 1956-1957 that occurred in individuals in Birmingham, England. Three hundred and twenty five infants under 2 years of age were admitted to the hospital with acute epidemic bronchiolitis. Supralaryngeal cough swabs were taken on admission from 87 cases for bacterial cultures. Before discharge from the hospital another swab was taken from 44 of these children. Each swab was cultured on blood agar and chocolate agar plates. These were incubated overnight at 37^{o} C. Seventy-five percent of these 44 children showed a change in their pharyngeal bacterial flora. Fifty percent of the potentially pathogenic organisms isolated on admission were reisolated from the same children on discharge, and similarly 50 percent of the pathogenic organisms isolated on discharge were present on admission. These investigators

reported that the potentially pathogenic organisms found in the cough swabs were not related to the clinical condition, and that the cultural patterns in any particular child appeared to change regardless of therapy. In some cases where death occurred, postmortem examinations were performed. The bronchi and bronchioles were congested with thick mucus. Scattered throughout all lobes were dark reddish-purple unaerated areas and patches of emphysema with varying degrees of pulmonary edema. In addition to the above findings, there were areas of consolidation and abscesses typical of staphylococcal pneumonia. Virology studies were conducted on 73 children during the winter of 1956-1957. Throat swabs, paired sera, feces and lung secretions, aspirated by syringe, were collected from these patients. From 8 fatal cases lung tissue or lung juices and tracheal secretions were obtained. The specimens were cultured in amniotic cavities of chick embryos and on monolayer cultures of HeLa, monkey kidney and human amnion cells. Complement fixation tests were performed on the paired sera for the presence of antibodies to influenza A, B, C, parainfluenza I, Psittacosis-lymphogranuloma venereum, Q-fever and adenoviruses. A small number of tests were attempted using parainfluenza II and III antigens. Neutralization tests against adenovirus type I were also used. Eight of 73 cases showed evidence of adenovirus infections. In 6 of these cases viruses were isolated, while 2 showed only

serologic evidence of virus infection. No viruses were isolated from the postmortem materials.

Viral studies in chronic bronchitis were investigated by Jack and Gandevia (1960). Nineteen patients with chronic bronchitis were observed for a year. Sixty two gargle specimens were obtained at the onset of symptoms referable to the upper respiratory tract. One hundred and thirty eight blood samples, including "acute" and "convalescent" specimens as well as regular quarterly blood samples were also obtained. Virus isolations and serologic techniques failed to reveal evidence of infections with influenza A and B, adenoviruses, hemadsorption or croup-associated viruses during the year of observation, in spite of clinical evidence of exacerbations of bronchitis. However, the presence of antibodies of influenza A and B and to adenovirus in about half of the patients reflected past experience with these viruses, but no evidence of a rising complement fixation titer indicative of recent infection was found.

Although the respiratory infection may initially be caused by viruses, patients who are usually admitted to the hospital may be already secondarily infected with bacteria. In these cases the problem is to control the secondary bacterial infections that may be caused by organisms not normally considered pathogens. May (1953) investigated the reliability of cultures from a single

specimen of sputum from 14 patients. Each of 14 patients were coughing up consistently purulent or mucopurulent sputum. A pathogen was isolated in the first specimen examined in 11 cases, and in 2 cases pathogens were found in the next expectorated sputum. In the remaining case a pathogen was found in the 6th specimen. The pathogens isolated from all of these sputa were either H. influenzae or D. pneumoniae or both. In one patient, the second specimen expectorated 10 minutes later contained large numbers of H. influenzae. Throat swab specimens were collected from 28 patients whose sputa were examined. Potentially pathogenic organisms were found in the sputum of 23 patients, but the same organisms could be isolated from the throats of only 12 of these patients. In other 4 patients in whom pathogens were present in the sputum, only in one patient was the pathogen also found in the throat. In one other patient a pathogen was isolated from the throat but not found in the sputum. The same non-pathogenic organisms were in the sputum and throat swab of each of the 28 patients investigated. May showed that a single culture from a specimen of sputum often fails to reveal all the species of bacteria present even though the culture is made from the purulent material. He suggested that a homogenized suspension could be prepared from which only one culture would be required to reveal all the species of bacteria present. He also stated that potential pathogens in the

respiratory tract, such as pneumococcus and H. influenzae are significantly related to the presence of pus in the sputum. Proof that these organisms are pathogens in chronic bronchitis depended on the demonstration that their removal from the sputum by chemotherapy was associated with the improvement in the patient's condition.

The viral studies of the upper respiratory diseases have shown that viruses were the causative agents of this disease and that the more commonly isolated viruses were the influenza A and B which was followed by adenoviruses. The viral studies of the lower respiratory diseases showed that in bronchiolitis adenoviruses were isolated from 6 of 73 infants, while the viral study in bronchitis revealed no isolation of virus. Most pathologists believed that viruses are the initial cause of chronic bronchitis because in their postmortem examinations of the lungs from patients they have found epithelial lesions typical of viral infection (Hers and Mulder, 1953, McLean, 1958a, 1959). However, virologists have attempted to isolate viruses from similar postmortem materials and antemortem lung secretions without success. There are very few studies of the viral diseases of the lower respiratory tract and the paucity of virus isolations may be related to (1) the small number of studies; (2) viral agents causing these diseases are not culturable by the methods employed; or (3) non-susceptible host tissue culture cells have been used.

MATERIALS AND METHODS

I. Method of obtaining saliva and sputum.

Patients from the Pulmonary Disease Service of the Veterans Administration Hospital, Salt Lake City, Utah were utilized in this study. An initial selection of patients known to have bronchitis, bronchiectasis, or pulmonary emphysema and who had not had antibacterial chemotherapy for 6 months were chosen by the Chief of the Pulmonary Disease Service and resident physicians.

The patient was required to brush his teeth thoroughly. He then rinsed his mouth for five minutes with sterile distilled water. If the patient wore dentures, these were removed and the gums were brushed in the same manner. He then rinsed his mouth for five minutes. After the five minute rinsing the patient was instructed to produce saliva by rotating his tongue in his mouth. The patient was repeatedly cautioned not to cough. At least 2 ml of saliva were collected in a sterile petri dish.

The patient again rinsed his mouth for two minutes with sterile distilled water. After this procedure the patient was instructed to cough up with as little saliva contamination as possible. Patients who could not cough up sputum were instructed to lie on the bed with their head down for best postural drainage. Sputum was allowed to remain in the mouth. If macroscopic examination of the sputum showed no mucous material typical of sputum, the specimen was discarded and the procedure repeated. At least 2 to 3 ml

of sputum were collected per specimen. Table I lists the patients who were utilized in this study.

TABLE I

PATIENT'S HISTORY

Patient's No.	Patient's initial	Age	Race	Diagnosis	Length of Illness (yr)
1	T.B.	31	W	1. Chronic bronchitis 2. Bronchiectasis, lingula, probable very mild 3. Post-thoracotomy, right middle lobe	28
2	C.B.	70	W	1. Chronic bronchitis	4
3	D.H.	64	W	1. Bullous emphysema 2. Chronic bronchitis	Many years
4	J.D.B.	61	W	1. Chronic bronchitis 2. Emphysema 3. Pulmonary tuberculosis, inactive	6
9	R.L.	46	W	1. Chronic bronchitis	8
10	I.G.S.	60	W	1. Pulmonary fibrosis, etiology unknown 2. Emphysema 3. Chronic bronchitis	8
11	P.M.	48	W	1. Pulmonary emphysema 2. Chronic bronchitis	1
12	A.S.D.	54	W	1. Pulmonary emphysema-bullous 2. Chronic bronchitis	1 1/2-2
13	H.N.	69	W	1. Pulmonary emphysema 2. Chronic bronchitis	3

TABLE I (continued)

Patient's No.	Patient's initial	Age	Race	Diagnosis	Length of Illness (yr)
14	H.D.	46	W	1. Pulmonary emphysema 2. Atopic asthma, probable	15
15	E.T.H.	70	W	1. Chronic bronchitis 2. Emphysema 3. Probable allergic component	9
16	F.S.	65	W	1. Emphysema, pulmonary due to unknown cause 2. Recurrent left pleural effusion of unknown etiology 3. Chronic bronchitis	3-4
17	W.B.C.	52	W	1. Obstructive pulmonary emphysema 2. Bronchitis 3. Bronchiectasis, rt. lower lobe; treated, improved	4
18	W.I.	38	W	1. Chronic bronchitis with minimal bronchiectasis 2. Solitary nodule in left upper lobe, most compatible with healed histoplasmosis	3 months
19	W.G.	57	W	1. Chronic bronchitis 2. Pulmonary emphysema	13
20	J.H.P.	65	W	1. Chronic bronchitis 2. Mild pulmonary emphysema 3. Arrested tuberculosis	1
21	J.L.	42	W	1. Pneumonitis, right lower lobe, etiology undetermined, treated, recovered. 2. Residual pleural thickening following pleural effusion and decorticated on right, remote	6

TABLE I (continued)

Patient's No.	Patient's initial	Age	Race	Diagnosis	Length of Illness (yr)
22	R.N.	25	W	1. Bronchiectasis of basilar segments of left lower lobe 2. Acute bacterial bronchial pneumonia, treated, improved	8
23	W.S.	65	W	1. Chronic bronchitis 2. Cor pulmonale 3. Diffuse pulmonary emphysema	5-6
24	J.C.H.	54	W	1. Chronic bronchitis 2. Possible viral pneumonitis 3. Possible bronchiectasis	10
25	R.C.S.	69	W	1. Bronchitis 2. Bronchiectasis 3. Pulmonary emphysema 4. Asthma	15
26	W.M.K.	57	W	1. Obstructive emphysema 2. Chronic bronchitis	15
27	G.H.	22	W	1. Probable bronchiectasis involving basilar segments of both lower lobes	5
28	C.R.	65	W	1. Bronchiectasis, all basilar segment of left lower lobe 2. Lingular, with minimal cylinderical bronchiectasis in upper lobe on the left side 3. Chronic bronchitis 4. Chronic rhinitis	Several yrs.
29	A.W.	61	W	1. Old apical pulmonary fibrosis, probably due to acid-fast disease, inactive 2. Present with some deviation of left hilar structure	4-5

TABLE I (continued)

Patient's No.	Patient's initial	Age	Race	Diagnosis	Length of Illness (yr)
30	J.E.	61	W	1. Mild emphysema 2. Bronchiectasis, involving basilar segments of the left lower lobe and basilar segment of right lower lobe in addition to the medial segment of the right middle lobe. 3. Chronic bronchitis	10

II. SALIVA

A. Qualitative bacterial count.

The saliva specimens were inoculated on 2 blood agar plates, 1 chocolate agar plate and thioglycollate broth. One blood agar was cultured aerobically and the other plate was cultured anaerobically. Both plates were incubated at 37° C for 48 and 72 hours respectively. The chocolate agar plate was incubated in a CO_2 jar at 37° C for 48 hours. The thioglycollate broth was incubated for 7 days at 37° C.

B. Quantitative bacterial count.

Dilutions of the saliva in 9 ml of saline were made starting with an initial 1:10 dilution with serial 10-fold dilutions through 6 tubes. The pour plates were prepared in duplicate using 1 ml each of 10^{-4}, 10^{-5}, and 10^{-6} dilutions. These plates were incubated at 37° C for 48 hours.

C. Animal inoculation.

Two young adult white mice were inoculated intraperitoneally,

one with 0.25 ml of saliva and the other with 0.5 ml of 1:10 dilution of saliva.

III. SPUTUM

A. Qualitative bacterial count.

The appearance of the sputum was observed and recorded. The appearance of these specimens was classified according to Douglas et al. (1957): (1) purulent, (2) muco-purulent, (3) containing traces of pus, and (4) mucoid. Cheesy or mucopurulent portions of the sputum were inoculated on blood agar and chocolate agar plates. The blood agar plates were incubated at 37° C for 48 hours and the chocolate agar plates were incubated in a CO_2 jar at 37° C for 48 hours. Direct smears of the sputum were made and gram stained. The sputum was homogenized repeatedly by drawing the sputum into a tuberculin syringe and rapidly expelling it. The homogenized sputum was cultured on 2 blood agar plates, 1 chocolate agar plate, and in thioglycollate broth culture. One blood agar plate was incubated for 48 hours at 37° C aerobically and the other blood agar was incubated for 72 hours at 37° C anaerobically. The chocolate agar plate was incubated under CO_2 tension for 48 hours at 37° C. The thioglycollate broth was incubated for 7 days at 37° C.

B. Quantitative bacterial count.

Dilutions of the homogenized sputum in 9 ml of 0.9 percent NaCl solution were made starting with an initial 1:10 dilution with serial 10-fold dilutions through 6 tubes. The pour plates were prepared in duplicate using 1 ml each of 10^{-4}, 10^{-5}, and 10^{-6} dilutions. These plates were incubated at 37^{o} C for 48 hours.

C. Animal inoculation.

Two young adult white mice were inoculated intraperitoneally, one with 0.25 ml of sputum and other with 0.5 ml of 1:10 dilution of sputum.

IV. METHODS OF SPUTUM STERILIZATION

A. Determination of the number of bacteria in the original sputum.

Twenty-four hour sputum specimens were collected from patients with chronic lung diseases who were not on chemotherapy. These specimens were homogenized in a microwaring blendor for 2 minutes. The number of bacteria in the non-sterile sputum was determined by diluting the sputum from 10^{-1} to 10^{-6}. Two percent blood trypticase agar pour-plates were prepared in duplicate using 1 ml aliquots of the 10^{-6} dilution.

B. Methods of sterilization

1. Heat

Fifty ml of homogenized sputum were placed in 63^{o} C waterbath.

At a specified time 2 ml samples were withdrawn and cultured by the pour-plate method mentioned above. These plates were incubated for 48 hours at 37° C and colonies were counted.

2. Ethylene oxide (C_2H_4O).

Fifty ml of homogenized sputum were sprayed several times with ethylene oxide and placed in a 37° C incubator. At a specified time 2 ml samples were withdrawn and cultured by the pour-plate method. Colonies were counted after 48 hours incubation at 37° C.

The residual ethylene oxide was removed by (1) putting the sputum specimen in a sterile suction flask, (2) allowing air to pass through the specimen, and (3) removing the gases by suction from the upper side arm of the flask.

3. Penicillin.

Five million units of Potassium penicillin-G were added to 50 ml of sputum. This mixture was incubated at 37° C. Aliquots were withdrawn at various time intervals. A number of bacteria were determined after 48 hours incubation at 37° C.

V. METHOD OF BACTERIAL GROWTH CURVE USING STERILE SPUTUM AS A MEDIUM FOR GROWTH

The organisms isolated previously from the patient's sputum were either grown in a trypticase soy broth or brain-heart infusion broth depending on the organism. The eighteen hour broth cultures were diluted with saline to obtain the initial optimal number of

bacteria necessary for pour plate count. Table II lists the organisms and the dilutions of the organisms.

TABLE II

Bacterial dilutions

Organisms	Dilutions in saline
Alpha hemolytic Streptococci	$1:10^3$
Non-hemolytic Streptococci	$1:10^4$
Beta hemolytic Streptococci	$1:10^3$
Diplococcius pneumoniae	$1:10^3$
Hemolytic Staphylococcus albus	$1:10^5$
Non-hemolytic Staphylococcus albus	$1:10^5$
Hemolytic Staphylococcus aureus	$1:10^5$
Non-hemolytic Staphylococcus aureus	$1:10^5$
Hemophilus influenzae	$1:10^3$
Hemophilus parainfluenzae	$1:10^3$
Hemophilus hemolyticus	$1:10^3$
Neisseria species	$1:10^3$
Coliforms (6 to 8 hours)	$1:10^2$
Pseudomonas aeruginosa (6-8 hours)	$1:10^2$

Five tenth ml each of the above diluted cultures was added to 4.5 ml of sterile sputum and 4.5 ml of trypticase soy broth or 4.5 ml brain heart infusion broth (control). Immediately after the addition of the organisms, duplicate blood agar pour plates were

made using a 0.1 ml aliquot from each of the inoculated control and sterile sputum. The sputum and control broth cultures were incubated at 37° C and additional aliquots were withdrawn at 3, 6, 8, and 24 hours. Duplicate pour plates were prepared from each of these samples. All of these pour plates were incubated for 48 hours at 37° C. The colonies were counted on the Quebec counter.

For the determination of the growth of Hemophilus organisms, a modification of the above method was necessary. These organisms were first grown in a brain heart infusion broth with 1% Supplement B (Difco) for 48 hours. Then the broth culture was diluted 10^{-2} with saline. Five tenth ml of the diluted culture was added to 4.5 ml of sterile sputum and 4.5 ml of brain heart infusion broth containing 1% Supplement B (control). Immediately after the addition of the organisms, duplicate blood agar pour plates containing 1% Bacto Fildes enrichment (Difco) were made using a 0.1 ml aliquot from each of the inoculate control and sterile sputum. The sputum and control broth cultures were incubated at 37° C and additional aliquots were withdrawn at 3, 6, 8, and 24 hours. Duplicate pour plates containing 1% Bacto Fildes enrichment (Difco) were prepared from each of these samples. All of these plates were incubated at 37° C in 3 to 5% CO_2 tension for 48 hours before the number of bacteria was counted.

VI. SENSITIVITY DETERMINATION OF TENSIOMETER

Cat. No. 70545 CENCO Tensiometer (Central Scientific Co.) was used in the measurement of surface tension of broths and sputa. The instrument was equipped essentially of a sensitive specialized torsion balance with a platinum iridium ring (circumference of 6 cm.) and with a stirrup suspended from a light weight lever extending horizontally from the fine torsion wire. Force was applied by means of a worm gear, for the regulation of the torque, and was measured on a circular scale with vernier graduated in dynes.

Dilutions of the detergent (*hemo-sol®), 1 gm in 9 ml of trypticase soy broth were made starting with an initial 1:10 dilution with serial 10-fold dilutions through 10 tubes. The liquid to be tested was placed in a 20 ml beaker. The platinum ring was immersed in the fluid just below the surface. The force to withdraw the ring was measured in dynes per centimeter. Surface tension readings were made in duplicate using all dilutions.

Dilutions of sodium lauryl sulfate ($CH_3(CH_2)_{10}CH_2OSO_3Na$), 5 ml in 5 ml of trypticase soy broth were made starting with an initial 1:2 dilution with serial 2-fold dilutions through 7 tubes. The surface tension readings were made using all dilutions.

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*Chemical composition of hemo-sol was not available.

VII. SURFACE TENSION MEASUREMENTS OF SPUTA AND CULTURE BROTHS

One ml of various 24 hour broth cultures was added to 9 ml of sterile sputum and a similar amount to 9 ml of trypticase soy broth or nutrient broth. Immediately after the addition of the organisms, a 5 ml sample was withdrawn, placed in a 20 ml beaker, and the surface tension measured (0 day). The remaining 5 ml was incubated at 37° C for 3-4 days. At the end of the incubation period, the surface tension readings were made. All measurements were performed in duplicate.

The surface tension measurements were performed in a 25° C room. The beakers were acid cleaned and rinsed several times each with tap water and then distilled water. The platinum ring was washed with 95% ethyl alcohol twice and then once in ethyl ether.

EXPERIMENTAL RESULTS

Most studies of the sputum flora in patients with chronic bronchitis, bronchiectasis, and emphysema are based on bacteriological cultures of expectorated bronchial secretions. Contamination by nasopharyngeal microorganisms is inevitable, since bacteria known to be normal inhabitants of the pharynx are cultured from expectorated sputum. Bacteria isolated in this manner cannot be assumed to have significance in bronchial inflammation.

This study was designed to compare quantitatively and qualitatively the bacterial flora of expectorated bronchial secretions and saliva in patients with chronic bronchitis, bronchiectasis, or emphysema, in an effort to assess the importance of contamination of the expectorated bronchial secretions by saliva.

Table III is a tabulation of the results obtained from the average bacterial counts of saliva and sputum of 26 patients known to have bronchitis, bronchiectasis or emphysema and bacterial counts of saliva from 4 patients not known to have any form of pulmonary disease.

TABLE III

Pour plate (Bacteria/ml specimen)

Patient's No.	Saliva bacteria/ml	Sputum bacteria/ml
1	3.0×10^7	1.2×10^6
2	4.6×10^7	5.8×10^6

TABLE III (continued)

Patient's No.	Saliva bacteria/ml	Sputum bacteria/ml
3	1.1×10^8	1.8×10^7
4	2.1×10^8	1.1×10^8
5 (Normal individual)	1.6×10^8	
6 (Normal individual)	2×10^8	
7 (Normal individual)	2.9×10^8	
8 (Normal individual)	7.8×10^6	
9	5.2×10^5	1.0×10^6
10	2.0×10^8	3.3×10^6
11	1.0×10^8	1.2×10^6
12	4.5×10^6	1.4×10^7
13	1.8×10^6	1.6×10^6
14	3.3×10^7	1.9×10^6
15	5.0×10^7	2.4×10^5
16	2.6×10^7	2.5×10^5
17	2.3×10^7	2.1×10^6
18	1.4×10^6	6.3×10^5
19	2.0×10^7	6.6×10^5
20	3.3×10^7	8.1×10^7
21	4.0×10^6	6.9×10^6
22	6.7×10^6	7.8×10^6
23	1.6×10^7	1.3×10^6
24	4.7×10^6	1.3×10^6
25	9.2×10^6	5.5×10^7
26	1.6×10^7	8.2×10^6
27	9.0×10^6	1.4×10^7
28	4.4×10^7	2.2×10^6
29	3.4×10^7	4.4×10^6
30	2.3×10^8	1.4×10^8

The number of bacteria found in the saliva was higher than the counts from the corresponding sputum in 15 of 26 cases studied. Seven of 26 patients show similar results. Four of 26 cases showed lower bacterial counts in saliva than in sputum. There is no difference in the bacterial counts of saliva of patients with bronchitis or emphysema with that of normal individuals. Although the table shows that the number of bacteria in the saliva is only a 1 log difference with the corresponding sputum, there seems to be a higher percentage of this incidence.

Table IV is a tabulation of the results indicating the general bacterial flora of the saliva from 26 patients known to have bronchitis, bronchiectasis or emphysema. The 5 predominant organisms found in the saliva from these patients were alpha hemolytic Streptococci (white strain), non-hemolytic Streptococci (white strain), Neisseria flavesecens, Neisseria perflava, and non-hemolytic St. aureus.

TABLE IV

Bacterial species isolated from saliva in 26 patients

Bacterial species	No. of isolates	% of subjects
Alpha hemolytic Streptococci		
(white)	19	73
(grey)	11	42
Non-hemolytic Streptococci		
(white)	25	96
(grey)	7	27
Beta hemolytic Streptococci		
(Not group A)	5	19

TABLE IV (continued)

Bacterial species	No. of isolates	% of subjects
N. flava	1	4
N. flavescens	14	54
N. perflava	18	69
H. hemolyticus	10	38
H. influenzae	2	8
H. parainfluenzae	3	12
D. pneumoniae	7	27
Non-hemolytic St. albus	7	27
Hemolytic St. albus	3	12
Non-hemolytic St. aureus	18	69
Hemolytic St. aureus	4	15
E. coli	2	8
Intermediate coliforms	1	4
Ps. aeruginosa	1	4
Achromobacter Sp.	2	8
Proteus rettgeri	1	4
Corynebacterium hof mannii	2	8
Corynebacterium xerose	1	4

Table V presents the results of the bacterial species isolated from saliva specimens of 4 normal subjects. These data suggest that alpha hemolytic Streptococci, non-hemolytic Streptococci, N. flavescens, N. perflava, N. sicca and non hemolytic St. albus are flora of the nasopharynx.

TABLE V

Bacterial species isolated from saliva in 4 normal subjects

Bacterial species	No. of isolates	% of subjects
Alpha hemolytic Streptococci (white)	4	100

TABLE V (continued)

Bacterial species	No. of isolates	% of subjects
Non-hemolytic Streptococci (white)	4	100
N. flavescens	3	75
N. perflava	4	100
N. sicca	2	50
Non-hemolytic St. albus	3	75

Table VI presents a comparison of the numbers of bacterial isolates from mucopurulent flecks of sputum and homogenized sputum among the 26 patients. There was a consistent decrease in the number of isolates in the flecks of mucopurulent sputum.

TABLE VI

A comparison of the bacterial species isolated from mucopurulent flecks of sputum and homogenized sputum in 26 patients

	Bacterial species isolated from:	
	Flecks of sputum	Homogenized sputum
Alpha hemolytic Streptococci (white)	65*	73
(grey)	27	46
Non-hemolytic Streptococci (white)	65	85
(grey)	19	8
Beta-hemolytic Streptococci (Not Group A)	12	12
N. flava	4	8
N. flavescens	38	46

TABLE VI (continued)

	Flecks of sputum	Homogenized sputum
N. perflava	58	73
N. sicca	0	4
H. hemolyticus	42	46
H. influenzae	23	23
H. parainfluenzae	12	19
D. pneumoniae	58	65
Non-hemolytic St. albus	8	27
Hemolytic St. albus	12	8
Non-hemolytic St. aureus	46	50
Hemolytic St. aureus	19	30
E. coli	12	12
Intermediate coliforms	4	4
Pseudomonas aeruginosa	8	8
Achromobacter Sp.	0	4
C. hofmanni i	4	19
C. xerose	4	0
Saccharomyces Sp.	8	19
Candida albicans	4	4

*Percent of patients

A comparison of the bacterial species isolated from saliva, mucopurulent flecks of sputum and homogenized sputum in 26 patients is listed on Table VII. The presence in both sputum and saliva of alpha hemolytic Streptococci (white and grey strains), non-hemolytic Streptococci (white and grey strains), the Neisseria species, non-hemolytic St. aureus and non-hemolytic St. albus suggests a nasopharyngeal origin of these strains. The pneumococci, hemolytic

St. aureus, and the Hemophilus species were less frequently isolated from saliva alone, but were often isolated from the sputum alone, suggesting a bronchial origin for these organisms.

TABLE VII

A comparison of the bacterial species isolated from saliva, muco-purulent flecks of sputum and homogenized sputum in 30 patients

Patient's No.	Bacterial species				
	Alpha-hemo. Streptococci (White) a b c	Alpha-hemo. Streptococci (Grey) a b c	Non-hemo. Strept. (White) a b c	Non-hemo. Strept. (Grey) a b c	Beta-hemo Strept. (Not group A) a b c
1	+ + -*	- - -**	+ + -	- - -	- - -
2	+ + +	- - -	+ - +	- - -	- - -
3	+ + +	- - -	+ + +	- - +	- - -
4	- - -	+ + +	- - -	+ + +	- - -
5	+***		+		
6	+		+		
7	+		+		
8	+		+		
9	+ + +	- - -	+ + -	- - -	- - -
10	+ + +	- - -	+ + +	- - -	- - -
11	+ + +	- - -	+ + +	- - -	- - -
12	+ + +	- + -	+ + +	- - -	- - +
13	+ - -	+ + +	+ + -	- - -	- - -
14	+ + +	+ + +	+ + +	- - -	- - -
15	+ + +	- - -	+ + +	+ + -	- - -
16	+ + +	- - -	+ + +	+ - +	- - -
17	- + +	+ + -	+ + -	+ - -	- - -
18	+ + +	+ + +	+ + +	- - +	- - -
19	+ + +	- + +	+ + +	- - -	- - -
20	+ + +	- - -	+ + +	- - +	- - -
21	+ + +	+ + +	+ + +	+ - -	- - -
22	+ - +	- - -	+ + +	- - -	+ - -
23	+ + +	- - -	+ - -	- - -	+ - -
24	- + +	+ - -	+ + +	+ - -	- - -
25	- - -	+ + -	+ + +	- - -	+ + +

TABLE VII (continued)

Patient's No.	Bacterial species				
	Alpha-hemo. Streptococci (White) a b c	Alpha-hemo. Streptococci (Grey) a b c	Non-hemo. Strept. (White) a b c	Non-hemo. Strept. (Grey a b c	Beta-hemo Strept. (Not group A) a b c
26	+++	- + -	+ + -	+ - -	+ + +
27	- + -	+ + -	+ + +	- - -	+ + -
28	+ + -	+ - -	+ + +	- - -	+ - -
29	+ + +	- - -	+ + -	- - -	- - -
30	+ - -	+ + +	+ + +	- - -	- - -

a = Saliva b - Homogenized sputum c - Flecks of sputum

* = Growth ** = No growth *** Numbers 5 to 8 are normal individuals

TABLE VII (continued)

Patient's No.	Bacterial species											
	N. flava			N. flavescens			N. perflava			N. sicca			H. hemolyticus			H. influenzae		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
1	-	-	-	+	+	+	-	-	-				-	-	-	-	+	+
2	-	-	-	-	-	-	+	+	+				-	-	-	+	+	+
3	-	-	-	+	+	+	+	+	+				-	-	-	-	-	+
4	-	-	-	-	+	-	+	+	+				+	+	+	-	+	+
5				+			+			+								
6				+			+			-								
7				+			+			-								
8				-			+			+								
9	-	+	-	-	-	-	-	-	-				+	+	+	-	-	-
10	-	-	-	-	-	-	+	+	+				+	+	+	-	+	+
11	+	+	+	-	-	-	-	-	-				-	+	-	-	-	-
12	-	-	-	-	-	-	+	+	+				-	-	-	-	-	-
13	-	-	-	+	+	+	+	+	+				+	-	-	-	-	-
14	-	-	-	+	-	-	+	+	+				+	+	+	-	-	-
15	-	-	-	-	-	-	+	+	-	-	+	-	+	+	+	-	-	-
16	-	-	-	+	-	-	+	+	-				+	+	-	-	+	-
17	-	-	-	+	+	-	+	+	-				-	-	+	-	-	-
18	-	-	-	-	-	-	+	+	+				+	+	+	-	-	-
19	-	-	-	-	-	-	+	+	+				-	-	-	-	-	-
20	-	-	-	-	-	-	+	+	+				-	-	-	-	-	-
21	-	-	-	+	+	+	-	-	-				-	-	-	-	-	-
22	-	-	-	-	+	+	-	-	-				+	+	+	-	-	-
23	-	-	-	+	+	+	-	-	-				-	-	-	-	-	-
24	-	-	-	+	-	+	+	+	+				+	+	+	-	-	-
25	-	-	-	-	-	-	-	+	-				-	-	-	-	-	-
26	-	-	-	+	+	+	-	+	-				-	-	-	-	-	-
27	-	-	-	+	-	-	+	+	+				-	+	+	+	-	-
28	-	-	-	+	+	-	-	-	-				-	+	-	-	+	+
29	-	-	-	+	+	+	+	-	-				-	-	-	-	-	-
30	-	-	-	+	-	+	+	+	+				-	-	-	-	-	-

TABLE VII (continued)

Patient's No.	Bacterial species														
	H. parainfluenzae			D. pneumoniae			Non-hemo St. albus			Hemo St. albus			Non-hemo St. aureus		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
1	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
3	-	-	-	-	-	+	-	+	-	-	+	+	+	+	+
4	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-
5							-								
6							+								
7							+								
8							+								
9	-	+	-	-	+	+	-	-	-	-	-	-	+	+	+
10	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
11	-	-	-	-	+	-	-	-	-	-	-	-	+	+	+
12	-	+	-	-	-	-	-	+	-	-	+	+	+	+	+
13	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
14	-	-	-	-	-	-	+	+	-	+	-	-	+	+	+
15	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+
16	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-
17	-	-	-	-	+	+	+	-	-	+	-	-	+	+	-
18	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
19	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
20	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+
21	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+
22	-	-	-	+	+	+	-	-	-	-	-	-	+	+	+
23	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
24	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-
25	-	-	-	+	+	+	-	+	+	-	-	-	-	-	-
26	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-
27	+	-	-	-	+	+	-	-	-	-	-	-	+	-	-
28	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+
29	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-
30	-	-	-	-	+	+	-	-	-	-	-	-	+	-	-

TABLE VII (continued)

Patient's No.	Bacterial species									
	Hemo. St. aureus			E. coli			Inter. med. coliforms			Ps. Aeruginosa			Achromo bacter Sp.		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
1	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-
4	-	+	+	-	-	-	-	-	-	-	+	-	+	-	-
5															
6															
7															
8															
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
11	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
12	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
16	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
17	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
26	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
27	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE VII (continued)

Patient's No.	Bacterial species														
	Pr. rettgeri			C. hofmannii			C. xerose			Saccharomyces Sp.			C. albicans		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-
4	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
5															
6															
7															
8															
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	+	+	+	-	-	+	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+
23	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-
24	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table VIII and Table IX record the results of a comparison of the cultural technique and animal inoculation for the isolation of

D. pneumoniae from saliva and sputum respectively in 26 patients. D. pneumoniae were isolated by cultural methods in 7 of 26 patients while concomitant mouse inoculations yielded 10 positive isolations among the same patients' saliva. Of the 10 organisms, 4 were isolated from mice only while 1 organism was isolated from culture only. The D. pneumoniae were isolated by the cultural technique in 18 of 26 patients' sputa while concomitant mouse inoculations yielded 12 among the same patients. Seven pneumococci were isolated culturally from sputum containing traces of pus, 6 organisms from mucoid sputum, 3 from muco-purulent sputum, and 2 from purulent sputum.

TABLE VIII

A comparison of the cultural technique and animal inoculations for the isolation of D. pneumoniae from saliva in 26 patients

Patient's No.	"In vitro" isolation of D. pneumoniae			"In vivo" isolation of D. pneumoniae
	Aerobic	CO_2	Anaerobic	
1	-*	-	-	+**
2	-	-	-	+
3	-	-	-	-
4	-	-	-	-
9	-	-	-	+
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-

TABLE VIII (continued)

Patient's No.	"In vitro" isolation of D. pneumoniae			"In vivo" isolation of D. pneumoniae
	Aerobic	CO_2	Anaerobic	
15	-	-	-	-
16	-	-	+	+
17	-	-	-	-
18	-	-	-	-
19	-	-	-	-
20	-	+	+	+
21	-	+	-	+
22	-	+	-	+
23	-	-	-	-
24	-	+	+	-
25	-	+	-	+
26	-	-	-	-
27	-	-	-	-
28	-	-	-	-
29	+	+	+	+
30	-	-	-	+

* = No growth or no isolation of D. pneumoniae
** = Growth or isolation of D. pneumoniae

TABLE IX

A comparison of the cultural technique and animal inoculations for the isolation of D. pneumoniae from sputum in 26 patients

Patient's No.	Appearance of sputum	"In vitro" isolation of D. pneumoniae		"In vivo" isolation of D. pneumoniae
		Flecks	Homogenized	
1	Trace of pus	+*	+*	-**
2	Purulent	-	-	-
3	Trace of pus	+	-	-
4	Purulent	-	+	+
9	Mucoid	+	+	+
10	Muco-purulent	-	+	+
11	Trace of pus	-	+	-

TABLE IX (continued)

Patient's No.	Appearance of sputum	"In vitro" isolation of D. pneumoniae		"In vivo" isolation of D. pneumoniae
		Flecks	Homogenized	
12	Trace of pus	-	-	-
13	Trace of pus	-	-	-
14	Trace of pus	-	-	-
15	Muco-purulent	-	-	-
16	Purulent	+	+	+
17	Muco-purulent	+	+	+
18	Trace of pus	-	-	-
19	Trace of pus	-	-	-
20	Trace of pus	+	+	+
21	Mucoid	+	+	+
22	Trace of pus	+	+	+
23	Muco-purulent	-	-	-
24	Mucoid	+	+	-
25	Muco-purulent	+	+	+
26	Mucoid	+	+	-
27	Trace of pus	+	+	+
28	Trace of pus	+	+	-
29	Mucoid	+	+	+
30	Mucoid	+	+	+

* = Growth or isolation of D. pneumoniae
** = No growth or no isolation of D. pneumoniae

In order to examine the alteration of surface tension by micro-organisms in culture medium, it was necessary to carry out 2 preliminary experiments. The first experiment was to find a suitable method for the sterilization of sputum and the second experiment was to investigate which organisms would grow in this sterilized sputum. Heat, ethylene oxide, and potassium penicillin-G were used in the sterilization of sputum. In the beginning the sterilization by ethylene oxide and heat was used interchangeably; but

after a suitable technique was found to remove the residual ethylene oxide from the sterile sputum, this latter method gave the better results. Therefore, most of the experiments were performed with sputum sterilized with ethylene oxide.

The following organisms were able to multiply in sterile sputum: alpha hemolytic Streptococci (white and grey strains), non-hemolytic Streptococci (white and grey strains), beta hemolytic Streptococci (not Group A), D. pneumoniae, non-hemolytic and hemolytic St. aureus, E. coli, Klebsiella-Aerobacter Group, Intermediate coliforms, Ps. aeruginosa, and Proteus species. The Hemophilus species, the Neisseria species, hemolytic and non-hemolytic St. albus were not able to grow under these conditions.

Figure I is the curve obtained from the surface tension readings of trypticase soy broth to which had been added hemo-sol of various concentrations. This procedure was performed to determine the sensitivity of the tensiometer. The results show that from 10^{-10} to 10^{-6} and from 10^{-3} to 10^{-1} dilutions of detergent, there was a gradual decrease in the surface tension of trypticase soy broth. There was a sharp decrease in the surface tension from 10^{-6} to 10^{-3} dilutions of hemo-sol.

FIGURE I

SURFACE TENSION CURVE OF TRYPTICASE SOY BROTH WITH HEMO-SOL

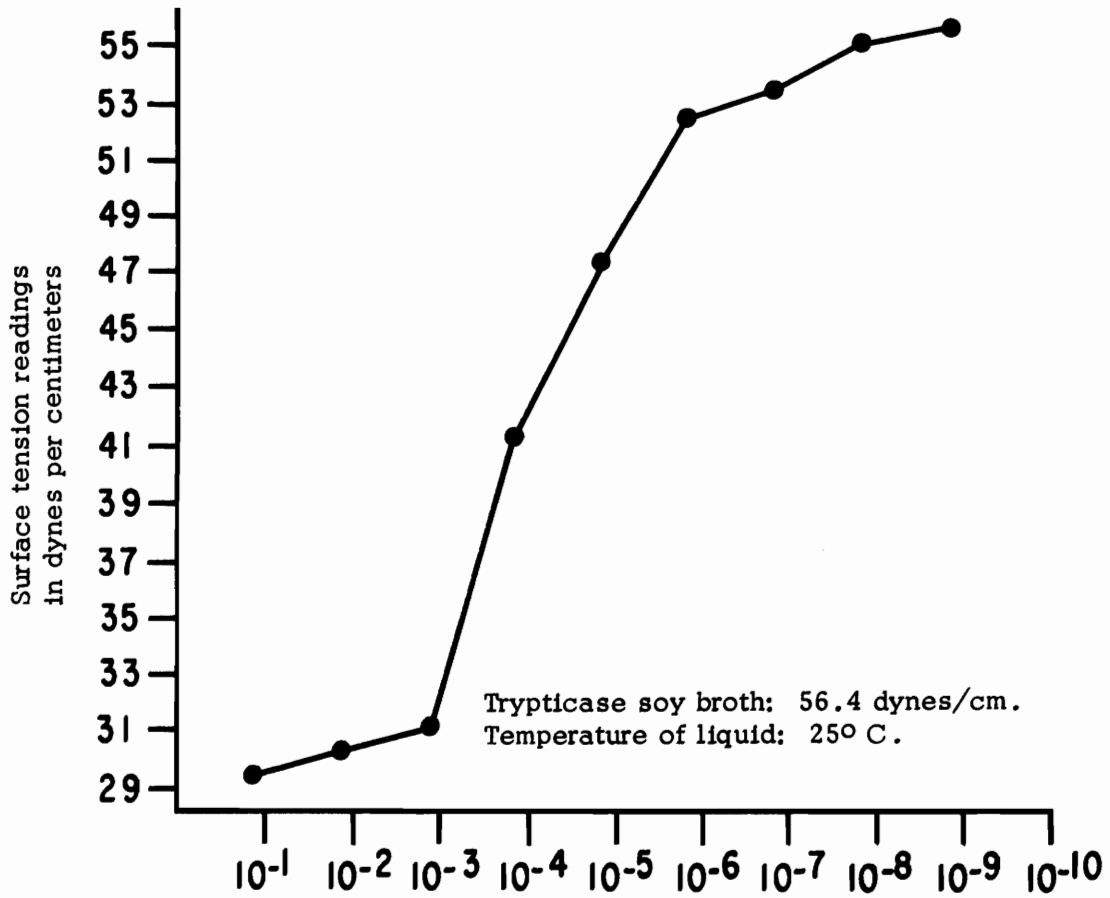
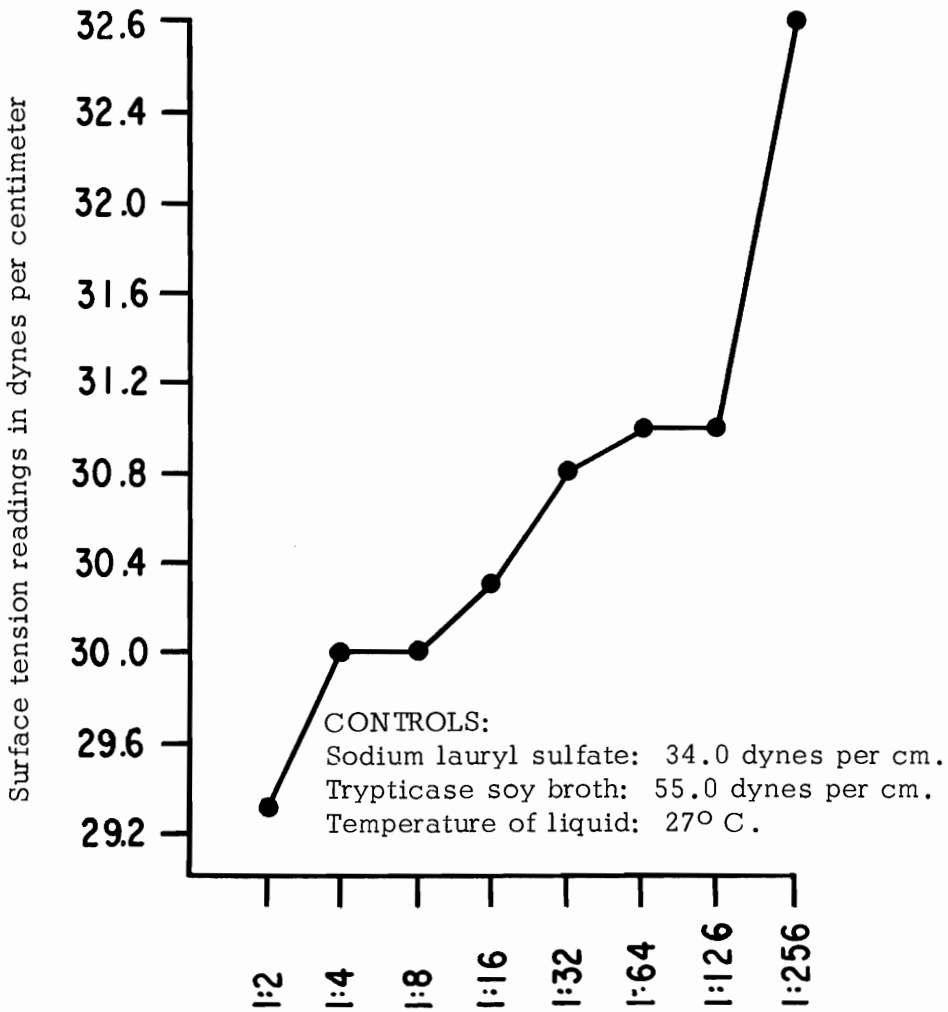


Figure II is the curve obtained from the surface tension readings of trypticase soy broth to which had been added sodium lauryl sulfate of various concentrations.

FIGURE II

SURFACE TENSION CURVE OF TRYPTICASE SOY BROTH WITH SODIUM LAURYL SULFATE



There was not much change in the reduction of surface tension of trypticase soy broth as the concentration of sodium lauryl sulfate was increased. From dilution of 1:2 up to 1:256, the change in the surface tension readings was only 3.3 dynes per centimeter.

Table X lists the results obtained from the average surface tension readings of trypticase soy broth and sputum in which organisms were added and allowed to grow. The readings were made on the broth and sputum before and after incubation with these organisms. An increase in the surface tension of trypticase soy broth was demonstrated by following organisms: alpha hemolytic Streptococci, non-hemolytic Streptococci (grey strain), beta hemolytic Streptococci, D. pneumoniae, hemolytic and non-hemolytic St. albus, non-hemolytic St. aureus, and Neisseria species. The surface tension of trypticase soy broth was decreased with hemolytic St. aureus, E. coli, Klebsiella-Aerobacter Group, and Ps. aeruginosa. The trypticase soy broth when incubated with the white strain of non-hemolytic Streptococci showed no change in surface tension.

TABLE X

The surface tension readings of trypticase soy broth and sputum containing organisms

Organisms	Trypticase soy broth		Sputum	
	0 Day dynes/cm	3-4 Days dynes/cm	0 Day dynes/cm	3-4 Days dynes/cm
Alpha hemolytic Streptococci (White)	53.4	57.1	45.5	44.8
Alpha hemolytic Streptococci (Grey)	49.8	54.7	45.2	43.0
Non-hemolytic Streptococci (White)	55.9	55.5	43.8	45.3
Non-hemolytic Streptococci (Grey)	53.6	59.6	44.6	46.5
Beta hemolytic Streptococci	57.3	58.7	47.1	47.3
D. pneumoniae	50.7	56.5	42.5	44.6
Hemolytic St. aureus	55.3	51.7	44.1	46.8
Non-hemolytic St. aureus	54.5	57.2	44.1	46.8
E. coli	53.5	48.2	45.4	48.3
Klebsiella-Aerobacter group	51.4	50.4	45	48.7
Ps. aeruginosa	55.0	40.2	44.0	47.1
Hemolytic St. albus	49.0	54.5	No growth	

TABLE X (continued)

Organisms	Trypticase soy broth 0 Day dynes/cm	Trypticase soy broth 3-4 Days dynes/cm	Sputum 0 Day dynes/cm	Sputum 3-4 Days dynes/cm
Non hemolytic St. albus	47.1	49.6	No growth	
Neisseria species	47.4	66.7	No growth	
Controls:				
Trypticase soy broth	51.3			
Sputum	42.0			

There was an increase in the surface tension of sputum with all organisms tested except the alpha hemolytic Streptococci which brought about a decrease and the beta hemolytic Streptococci which showed no change.

DISCUSSION

Although much work has been done by many investigators in the study of emphysema, the etiology as well as the pathogenesis of emphysema are still unknown. Therefore, a study was made to investigate the bacterial flora of patients with emphysema to see if there was a species or strain of organisms peculiar to emphysema. These bacteria were tested (1) for their ability to multiply in pulmonary mucus in vitro, (2) to examine the surface tension properties of mucus in vitro, and (3) to examine the alteration of surface tension of culture media by microorganisms.

A comparison of the numbers of bacteria in saliva and sputum in 26 patients with chronic bronchitis, bronchiectasis, and emphysema (Table III) showed that there were tremendous numbers of bacteria in saliva. Even "clean" mouth saliva contained as large or larger numbers of bacteria than did expectorated sputum. These bacterial counts indicate or suggest that more meaningful bacteriologic studies of sputum could be obtained if the patients' mouth were cleansed free of saliva.

The five predominant organisms found in the saliva from the 26 patients (Table IV) were alpha hemolytic Streptococci, nonhemolytic Streptococci, N. flavascens, N. perflava, and nonhemolytic St. aureus. These same organisms except the non-

hemolytic St. aureus were also found in the 4 normal individuals (Table V). Non-hemolytic St. albus was found in 75 percent and N. sicca were found in 50 percent of the normal subjects.

A comparison of the numbers of bacterial isolates from mucopurulent flecks of sputum and homogenized sputum among the 26 patients showed (Table VI) a slight but consistent decrease in the numbers of isolates of alpha hemolytic Streptococci, non-hemolytic Streptococci, and other non pathogens in the flecks of mucopurulent sputum. While the pneumococci and the Hemophilus species were isolated in equal numbers from the flecks and homogenized sputum.

The presence in both sputum and saliva of alpha hemolytic Streptococci, non-hemolytic Streptococci, the Neisseris species, non-hemolytic St. aureus, and non-hemolytic and hemolytic St. albus suggests a nasopharyngeal origin of these strains (Table VII). The pneumococci, hemolytic St. aureus and the Hemophilus species were less frequently isolated from the saliva alone, but were often isolated from the sputum suggesting a bronchial origin for these organisms. Pecora and Yegian (1959) have reported that bronchial secretions obtained by tracheal puncture from patients with chronic pulmonary disease are sterile. These authors believed that chronic infections of the lower respiratory tract with pyogenic bacteria are a rare occurrence and that sputum and secretions obtained at

bronchoscopy were contaminated by organisms normally present in the mouth. Laurenzi et al (1961) reported that bronchitis and bronchitis-associated-bronchogenic carcinoma patients have abundant bacterial flora in their lower respiratory tract. None of their bronchial secretions were free of oropharyngeal commensals. Out of 19 cases studied, there were 10 potential pathogens. H. influenzae and D. pneumoniae were found in 8 of 19 patients, St. aureus, coagulase positive in 5 patients, beta hemolytic Streptococci in one, E. coli in 5 patients. Specimens from the trachea and bronchi were taken by swabbing through the bronchoscope in 40 patients and through rubber catheters in 12 patients.

H. hemolyticus, a non-pathogenic gram negative bacillus often confused with beta hemolytic Streptococci was found in 46 percent of the patients. Previous investigators have not mentioned this organism. It may be that some investigators have included this organism with their H. influenzae or the parainfluenzae groups.

Anaerobic cultures were employed for the isolation of Bacteriodes species and other strict anaerobes. The organisms in the thioglycollate broth were subcultured aerobically and anaerobically. In all cases, no Bacteriodes or other strict anaerobes were isolated.

The isolation of D. pneumoniae by animal inoculation yielded more positive cultures than by cultural techniques using saliva (Table VIII). This difference in the numbers of isolation of this organism by the

two techniques could be due to the rare numbers of pneumococci present in the saliva. The organisms were able to grow and multiply *in vivo* but not as well in *in vitro*. The greater numbers of isolations of pneumococci by cultural technique from the sputum than by animal inoculation indicate that the strain was not pathogenic to mice (Table IX).

Pneumococci were isolated more frequently from mucoid sputum and sputum containing traces of pus (Table IX). Hemophilus species were isolated more frequently from sputum containing traces of pus. These findings are not in accord with that of Knox and Elmes (1955), Douglas *et al.* (1957), and Hallet *et al.* (1959). These investigators reported a high incidence of pneumococci and Hemophilus species in purulent and mucopurulent sputum.

The surface tension measurements of sputum were performed only when organisms were shown to be able to multiply in sterile sputum. The organisms able to multiply under such conditions were: alpha hemolytic Streptococci (white and grey strains), non-hemolytic Streptococci (white and grey strains) beta hemolytic Streptococci, *D. pneumoniae*, non-hemolytic and hemolytic *St. aureus*, *E. coli*, Klebsiella-Aerobacter group, *Ps. aeruginosa*, and Proteus species. The growth in sterile sputum may not necessarily indicate growth of organisms within the mucous plug in a patient, because sterilization procedures may cause chemical,

nutritional or physical changes in sputum.

Due to an epidemic of respiratory infections which occurred throughout the hospital, all patients were put on antibiotic therapy. The availability of sputa from patients who were not on antibiotics was thus limited. Because of the limited quantity of sputum, the surface tension readings were also limited.

This investigation has shown that the proliferation of alpha hemolytic Streptococci (white and grey strains), non-hemolytic Streptococci (grey strain), Beta hemolytic Streptococci, D. pneumoniae, non-hemolytic St. aureus, hemolytic and non-hemolytic St. albus, and the Neisseria species in trypticase soy broth (Table X) increased the surface tension of trypticase soy broth while the proliferation of hemolytic St. aureus, E. coli, Klebsiella-Aerobacter group, and Ps. aeruginosa decreased the surface tension of trypticase soy broth. There was no change in the surface tension of trypticase soy broth with non-hemolytic Streptococci (white strain).

The surface tension of sputum was increased with the growth and multiplication of non-hemolytic Streptococci (white strain), D. pneumoniae, hemolytic and non-hemolytic St. aureus, E. coli, Klebsiella-Aerobacter group, and Ps. aeruginosa in sputum (Table X). The proliferation of alpha hemolytic Streptococci (white and grey strains) in sputum decreased the surface tension of sputum. There was no change in the surface tension of sputum with beta hemolytic Streptococci.

SUMMARY

1. The bacterial flora and the number of bacteria in saliva and sputum of 26 patients with bronchitis, bronchiectasis, or emphysema were investigated.
2. The number of bacteria in the saliva and sputum taken from the same patient was compared.
3. The bacterial flora of the saliva and sputum taken from the same patient were studied aerobically, anaerobically, and under increased carbon dioxide tension.
4. The bacterial flora from mucopurulent flecks of sputum and homogenized sputum specimens were investigated.
5. The isolation of D. pneumoniae by animal inoculation was discussed.
6. The sterilization of sputum was investigated. Heat and ethylene oxide were used in the sterilization of sputum.
7. Sterile sputum was employed as a substrate for the possible growth of bacteria from patients with emphysema, and it was found that some of these bacteria grew well in sputum. These organisms were listed.
8. The surface tension of trypticase soy broth containing hemo-sol or sodium lauryl sulfate in varying concentrations was measured and reported.
9. The surface tension of trypticase soy broth and sputum was measured before and after the growth of microorganisms.

CONCLUSION

McLean (1959) observed mucous plugs containing great numbers of bacteria in microscopic sections of bronchioles. He postulated that the basic inflammatory disease in the bronchioles is due to the proliferation of these bacteria. Many investigators have reported the isolation of Pneumococci and H. influenzae from sputum or bronchial secretions and have implicated these organisms in bronchitis and bronchiectasis.

It is believed that one of the predisposing causes of emphysema is chronic bronchitis and bronchiectasis. Therefore, a study was made to investigate the bacterial flora of the saliva and sputum from 26 patients with chronic bronchitis, bronchiectasis, or emphysema. An high incidence of D. pneumoniae and Hemophilus species in sputum from these patients were isolated.

It has been further postulated by McLean (1958a, 1958b, 1959) that organisms which are able to remain for prolonged time in the respiratory tract may allow bacteria to multiply. Although he believed that virus infections were the causative factor in the bronchiolar damage, the secondary infections, especially those caused by "bacteria of low pathogenicity" were the cause of the bronchiolar inflammation and damage. If the mucous plugs were not expelled, the lumen may become partly or completely occluded. The bacteria which are multiplying in the mucous plugs can alter

the surface tension property of mucus and cause the bronchiolar wall adjacent to the plugs to lose its elasticity to a point that the contractive force is also lost. In this case, bronchiolar obstruction may occur.

This study has shown that several organisms were able to multiply in sterile sputum. The growth of bacteria in sputum *in vitro* may not necessarily suggest growth of organisms within the mucous plug in a patient, because sterilization procedures may cause chemical, nutritional or physical changes in sputum.

The investigation of surface tension of sputa showed that the proliferation of some microorganisms increases or decreases the surface tension of sputa depending upon the organism.

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BACTERIOLOGIC ASPECTS OF CHRONIC PULMONARY EMPHYSEMA

by

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ABSTRACT

Although much work has been done by many investigators in the study of emphysema, the etiology as well as the pathogenesis of emphysema are still unknown. Therefore, a study was made to investigate the bacterial flora of patients with emphysema, to see if there was a species or strain of organisms peculiar to emphysema. These bacteria were tested (1) for their ability to multiply in pulmonary mucus in vitro, (2) to examine the surface tension properties of mucus in vitro, and (3) to examine the alteration of surface tension of culture media by microorganisms.

Twenty-six patients from the Pulmonary Disease Service of the Veterans Administration Hospital, Salt Lake City, Utah were utilized in this study. An initial selection of patients known to have bronchitis, bronchiectasis, or emphysema and who did not have antibacterial chemotherapy for 6 months were chosen by the Chief of the Pulmonary Disease Service and resident physicians.

Most studies of the sputum flora in patients with chronic bronchitis and bronchiectasis are based on bacteriological cultures of expectorated bronchial secretions. Contamination by nasopharyngeal microorganisms is inevitable, since bacteria known to be normal inhabitants of the pharynx are cultured from expectorated sputum. Bacteria isolated in this manner cannot be assumed to have significance in bronchial inflammation.

This study was designed to compare qualitatively and quantitatively the bacterial flora of expectorated bronchial secretions and saliva in patients with chronic bronchitis or bronchiectasis, in an effort to assess the importance of contamination of the expectorated bronchial secretion by saliva.

A comparison of the numbers of bacteria in saliva and sputum in 26 patients showed that there are tremendous numbers of bacteria in saliva. Even in a "clean" mouth, saliva contained as large or larger numbers of bacteria than did expectorated sputum.

The presence in both sputum and saliva of alpha hemolytic Streptococci, non-hemolytic Streptococci, the Neisseria species, non-hemolytic Staphylococcus aureus and non-hemolytic and hemolytic St. albus suggests a nasopharyngeal origin of these strains. The D. pneumoniae,* hemolytic St. aureus, and the Hemophilus species were less frequently isolated from the sputum suggesting a bronchial origin for these organisms.

Besides using the cultural technique for the isolation of D. pneumoniae, white mice were inoculated with saliva and sputum. It was found that animal inoculation yielded more positive isolations than by cultural methods in saliva. However, there was a greater number of isolation of D. pneumoniae by cultural technique from the sputum than by animal inoculation.

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* Diplococcus pneumoniae

Sterile sputum was employed as a substrate for the possible growth of bacteria from patients with emphysema, and it was found that some of these bacteria grew well in sputum.

The surface tension measurements of sputa and broths were performed before and after incubation with organisms. This investigation has shown that the proliferation of microorganisms in sputum or broth either increased or decreased the surface tension of the sputum or broth depending on the bacteria.